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Granulocyte Colony-Stimulating Factor (G-CSF) Mediated Mobilization of Leukemic Cells in Philadelphia Chromosome Positive Acute Lymphoblastic Leukemia Expressing Myeloid Antigens (my^+Ph^+ALL)

Guido Kobbe,* Ullrich Bauser, Claudia Rieth, Ali Hunerliturkoglu, Dietmar Söhngen, Manuel Aivado, Carlo Aul, and Axel Heyll

Department of Hematology, Oncology and Clinical Immunology, Heinrich Heine University Duesseldorf, Duesseldorf, Germany

The therapeutic benefit of G-CSF in the treatment of acute lymphoblastic leukemia has been well established. G-CSF has been used to shorten neutropenia induced by conventional dose cytotoxic chemotherapy and allogeneic bone marrow transplantation. Recently autologous peripheral blood progenitor cell transplantation has been explored to treat high-risk ALL. Several in vitro studies suggest that subpopulations of lymphoblasts express G-CSF receptors. Furthermore, enhanced growth of Ph^+ ALL cells expressing myeloid antigens stimulated by G-CSF has been demonstrated in vitro. However, the clinical relevance of these findings has been questioned. We report a patient with my^+Ph^+ALL in whom the administration of G-CSF after high-dose Cytarabin and Mitoxantrone led to a significant mobilization of leukemic cells and contamination of the stem cell harvest during cytologic marrow remission. Am. J. Hematol. 58:330–333, 1998.

Key words: granulocyte colony-stimulating factor; acute lymphoblastic leukemia; myeloid antigen expression; peripheral blood progenitor cell transplantation

INTRODUCTION

Several randomized trials have demonstrated the therapeutic benefit of G-CSF administration after or even simultaneously with intensive cytotoxic treatment for acute lymphoblastic leukemia [1]. Patients receiving G-CSF after intensive chemotherapy experienced less toxicity in terms of febrile episodes, mucositis, and severe infections [2]. However, survival was not significantly influenced by G-CSF administration. G-CSF has been used with success to enhance the mobilization of haematopoietic progenitor cells following chemotherapy in various malignant diseases [3]. Mobilized progenitor cells have been used extensively to reestablish haematopoiesis after myeloablative therapy. This approach has recently been explored to treat Ph^+ ALL in patients lacking an HLA-identical stem cell donor [4]. Concern has been expressed about the potential effect of G-CSF on malignant myeloid and non-myeloid cells. Several in vitro studies have demonstrated the expression of G-CSF receptors on lymphoblasts, but the clinical relevance of this finding has been questioned [5]. We report a case of my^+Ph^+ALL where G-CSF administration after intensive chemotherapy during complete marrow remission was associated with a dramatic increase of leukemic cells in the peripheral blood and a significant contamination of the peripheral blood progenitor harvest.

CASE REPORT

A 32-year-old man was diagnosed having acute lymphoblastic leukemia in June 1997. Flow cytometric analysis of a bone marrow sample demonstrated 98% infiltration by lymphoid blasts expressing CD34, CD19, CD10, HLA-DR, CD15, CD33, TdT, and cyCD22. Myeloperoxidase staining on bone marrow smears was

*Correspondence to: G. Kobbe, M.D., Department of Hematology, Oncology and Clinical Immunology, Heinrich Heine University Duesseldorf, Moorenstr. 5, 40225 Duesseldorf, Germany.

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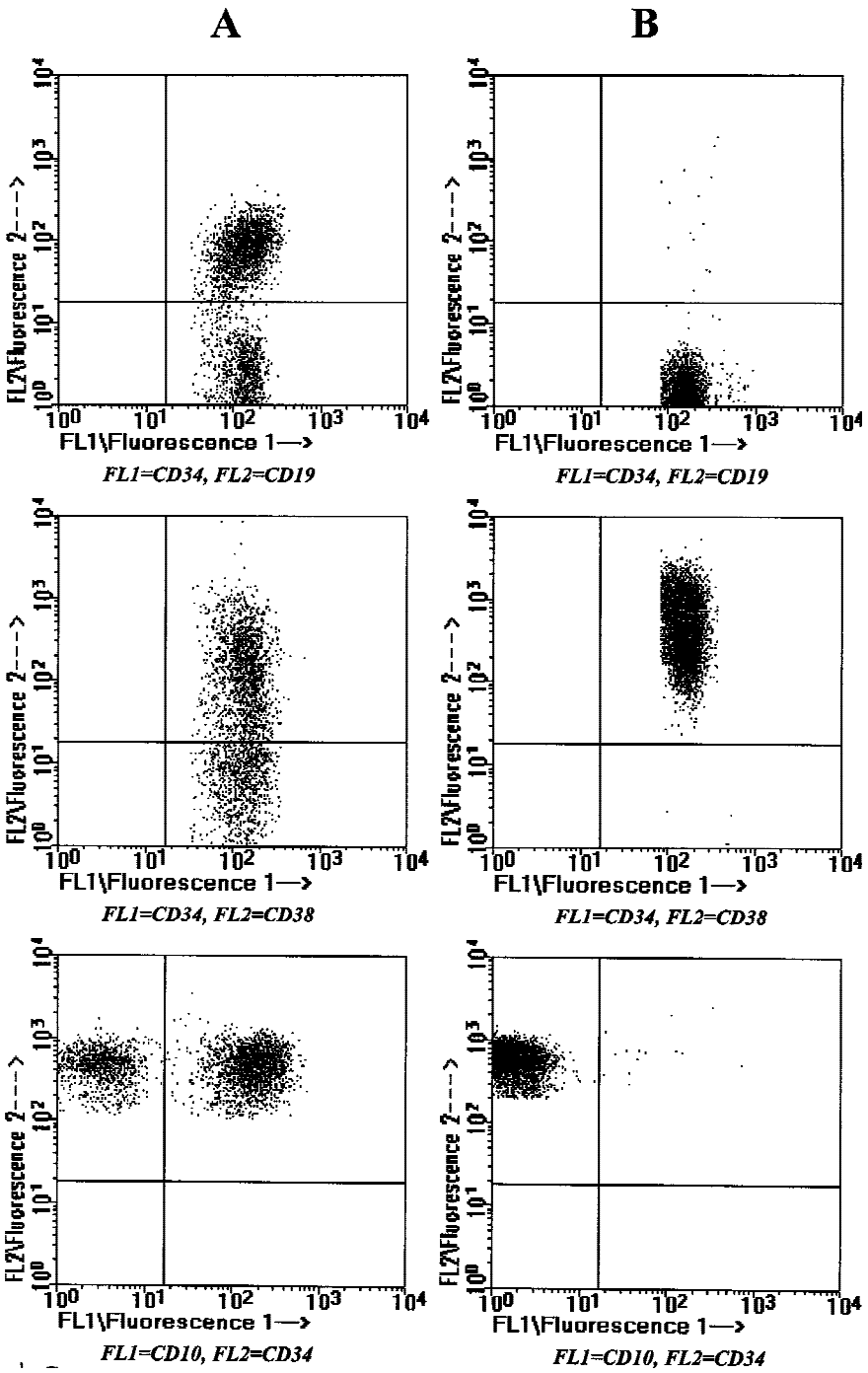

Fig. 1. Representative plots from two peripheral blood stem cell harvests showing coexpression of CD19 and CD10 and abnormal expression of CD38 on CD34$^+$ cells from the patient under discussion (A) and absence of CD19 and CD10 on CD34$^+$ cells from a control patient (B, patient with a germ cell tumor mobilized with chemotherapy and G-CSF).

negative. Cytogenetic and molecular studies were positive for the Philadelphia chromosome (t9,22). The patient was treated according to the current German Multicenter ALL therapy protocol and achieved complete cytologic marrow remission following treatment with prednisolone, vincristine, L-asparaginase, and daunorubicin. Fifty-two days after diagnosis, the patient received high-dose cytarabine and mitoxantrone (HAM, Cytarabin 2 × 3 g/m^2, days 1–4, and mitoxantrone 10 mg/m^2, days 3–5) followed by G-CSF (Filgrastim, Amgen, Munich, Germany, 480 μg/d subcutaneously). A period of 18 days of neutropenia was followed by a rapid recovery of the peripheral white blood count. Flow cytometry discovered a total of 303 CD34$^+$ cells per microliter peripheral blood on day 20 after HAM. Large volume leukapheresis was initiated immediately and yielded a total of 26.2 × 10^6 CD34$^+$ cells per kilo. Surprisingly the majority of the CD34$^+$ cells expressed CD19, CD10, CD33, and CD13 (Fig. 1). Detailed flow cytometric analysis of the harvest demonstrated the leukemic phenotype (expression of

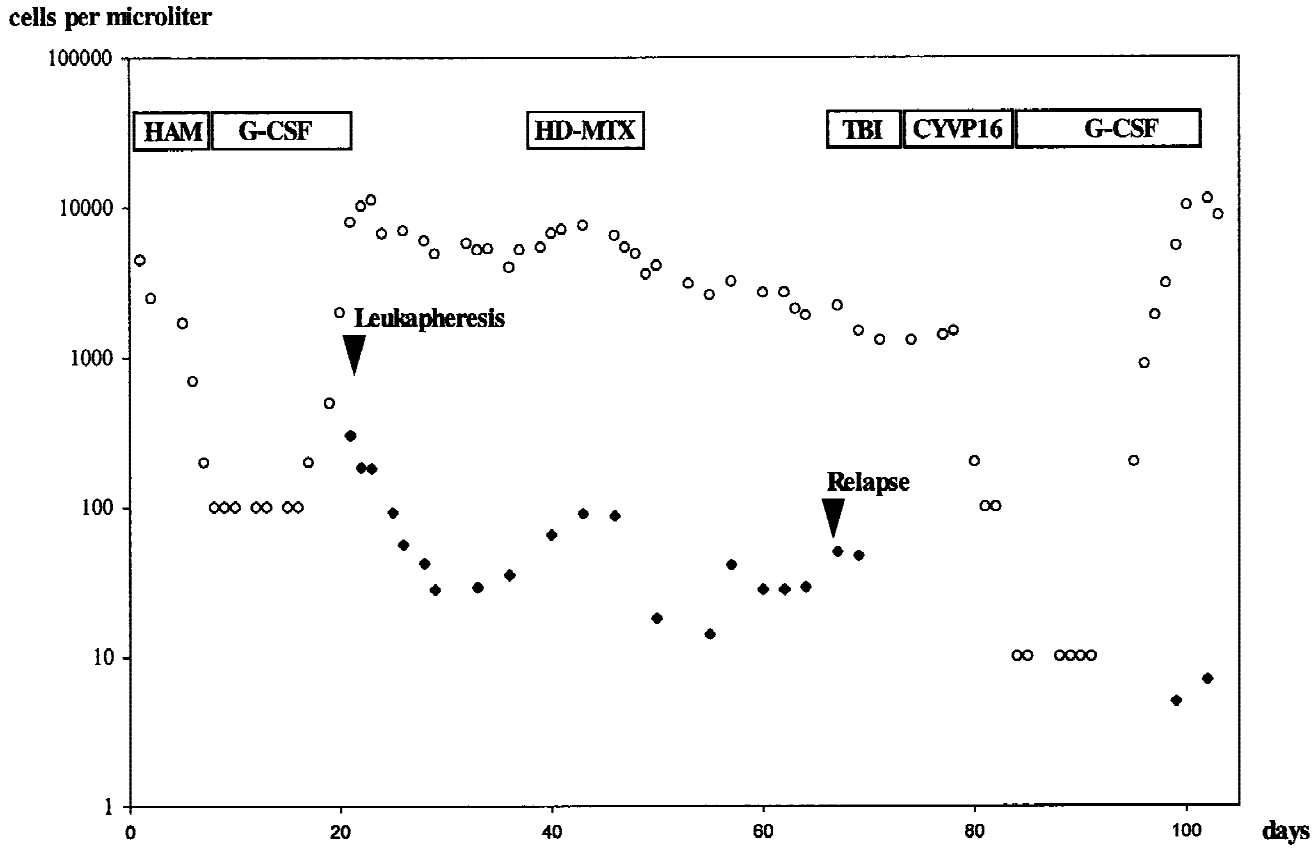

Fig. 2. Peripheral levels of leukocytes (○) and CD34+ cells (♦). Peripheral CD34+ cell counts (coexpressing CD19, CD10, CD13 and CD33) were highest during G-CSF administration following high-dose Ara-C/Mitoxantrone (HAM) and decreased within a few days after cessation of G-CSF. High-dose MTX decreased peripheral CD34+ cell counts only marginally. Without G-CSF administration peripheral CD34+ cell counts remained below 100/μl even during overt marrow relapse. Following allografting, mobilized CD34+ cells were not expressing CD19 and CD10.

CD34, CD19, CD10, HLA-DR, CD13, CD33) in 64% of the $CD34^+$ cell fraction. Polymerase chain reaction (PCR) for the bcrabl transcript was positive. Bone marrow cytology showed massive myeloid hyperplasia with lymphoblast representing less than 2% of the nucleated marrow cells. Flow cytometry discovered the leukemia phenotype in 3% of the MNC fraction. G-CSF was discontinued and the peripheral $CD34^+$ cell count decreased to 30 $CD34^+$ cells per microliter within a few days (Fig. 2). During the following weeks, the peripheral level of $CD34^+$ cells remained relatively stable and was only marginally influenced by further therapy (high-dose MTX, mercaptopurin, asparaginase). The patient remained in cytologic marrow remission until about 9 weeks after HAM when lymphoblasts represented 20% of the nucleated bone marrow cells. Two days later he received hyperfractionated total body irradiation (12 Gy), etoposid (40 mg/kg), and cyclophosphamide (120 mg/kg) followed by transfusion of G-CSF mobilized peripheral blood progenitor cells from an HLA-matched unrelated donor. During G-CSF supported recovery, $CD34^+$ cells were again mobilized into the circulation. However, now more than 90% of the circulating $CD34^+$ cells were negative for CD19 and CD10.

DISCUSSION

It seems very likely that G-CSF following HAM mobilized large numbers of leukemic cells in our patient. Peripheral counts of $CD34^+/CD19^+/CD10^+/CD33^+$ cells were highest during G-CSF administration and decreased dramatically upon cessation of the haematopoietic growth factor at a time when the numbers of peripheral granulocytes were still rising. A low level of circulating leukemic cells persisted over the following weeks. Without G-CSF stimulation, peripheral $CD34^+$ counts remained below 100/μl even during overt marrow relapse shortly before allografting. The fact that the circulating tumor cells were not significantly decreased by HAM or HD-MTX suggests a high level of drug resistance. However, supralethal radiochemotherapy depleted the leukemic phenotype below the limit of flow cytometric detection although PCR for the bcrabl fusion transcript remained weakly positive until 3 weeks after allografting.

Several groups have recently explored the use of autologous peripheral blood progenitor transplantation in patients with Ph^+ acute lymphoblastic leukemia. Clinical results so far are not encouraging because the majority of patients relapse early [4]. Often cytogenetic studies fail to demonstrate the Philadelphia chromosome in the leukapheresis product although the bcrabl fusion transcript can be detected by a sensitive PCR technique in most cases. Flow cytometric studies can help to identify the contamination of autologous grafts by $CD34^+/CD19^+/CD10^+$ blasts because, in contrast to bone marrow, this phenotype is rare among mobilized $CD34^+$ cells [6]. G-CSF receptors have been demonstrated on a high percentage of ALL blast with myeloid antigen expression [7]. Especially Ph^+ blasts with myeloid antigen expression have shown in vitro response to G-CSF [5]. This proliferative response to G-CSF has been used to enhance the susceptiblity of my^+ ALL blast to cytotoxic therapy [8]. Besides inducing proliferation in subsets of ALL, G-CSF may also be involved in the modification of adhesion characteristics of my^+ ALL blasts resulting in tumor cell mobilization. To our knowledge the patient under discussion represents the first case where a significant mobilization of acute lymphoblastic leukemia blasts by G-CSF has been demonstrated. The case is especially worrying because the patient received highly effective chemotherapy before G-CSF administration. Similar effects may be responsible for occult contamination of peripheral blood stem cell harvests in other cases of high-risk ALL. Consequently, sensitive immunologic and molecular studies should be utilized to detect tumor cell mobilization and harvest contamination in cases of ALL undergoing autotransplantation. Furthermore, careful clinical trials are needed to determine the relevance of graft contamination.

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